Journal of Research on the Lepidoptera 19(4): 181-195, 1980(81)

Enzyme Electrophoretic Studies on the Genetic Relationships of Pierid Butterflies (Lepidoptera: Pieridae) I. European Taxa*

H. J. Geiger

Zoologisches Institut der Universitat Bern, Sahlistrasse 8, CH 3012 Bern, Switzerland

Abstract. The phylogenetic relationships of 24 taxa representing the four European subfamilies of the Pieridae were studied using enzyme electrophoretic techniques. The mobilities of 20 enzymes, each determined by a different locus, were compared. A dendogram is presented indicating the grouping of the taxa on the basis of biochemical data. Results agree well with those obtained from conventional systematic studies. In cases where the relationships between taxa are under discussion, one of the alternatives is supported. The Pierinae and Anthocharinae are biochemically clearly characterisable entities although the branching point of these two subfamilies in the dendogram lies only slightly below those of different genera. These two subfamilies are separated from the Coliadinae by a clear step of genetic divergence. Within the recently subdivided genus *Pieris* Shrank s. ltr. of the Pierinae, three groups of taxa that branch at the same level of similarity in the dendogram can be recognized. The taxa of the new genus *Artogeia* Vrty. are no more closely related to each other than they are to the taxa of *Pieris* s. str. Biochemical data support the species rank of the taxa *cheiranthi* Hbn., *simplonia* F. and *crameri* Btlr., but indicate a remarkably low level of genetic differentiation between the taxa *napi* L. and *bryoniae* Hbn.

Introduction

The systematics of pierid butterflies have been extensively studied by several independent taxonomic methods and the Pieridae are now one of the best known butterfly families. Despite this, diverging views exist on the relationships and status of several taxa at different hierarchical rank. Systematic problems at the levels of 1) subfamilies and tribes, 2) genera, subgenera and species groups and 3) species and subspecies are discussed below.

1) In a generic revision of the family, Klots (1931/32) grouped the Pieridae into three subfamilies: Pseudopontiinae, Dismorphiinae and Pierinae and subdivided the Pierinae into three tribes: Euchloini, Rhodocerini and Pierini. This classification, with or without modifications, has

*This study was supported by the Swiss National Science Foundation Grant No. 3.640.80.

been followed by virtually all subsequent authors. In a comparative analysis of the morphology of the Papilionoidea, Ehrlich (1958), following other authors (e.g. Ford, 1945), elevated the Coliadinae (Rhodocerini sensu Klots, 1931/32) to subfamily status. This classification reflects the closer morphological relationship between the Pierini and Euchloini than between these two tribes and the Coliadinae or Dismorphiinae. Higgins (1975) adopted this system for the European Pieridae, but gave the Anthocharinae (Euchloini sensu Klots) subfamily rank. In the same work, Higgins included the genus *Catopsilia* Hbn. in the Pierinae in the tribe Callidryini Kirby but most other authors, including Klots (1931/32), have regarded this genus as best placed in the Coliadinae.

2) Of the European taxa, species of the genus *Pieris* Schrank have been especially problematical. This genus has long been recognized as being globally distributed and various authors have included a great number of taxa in it, (e.g. Roeber, 1908). In his revision of the Pieridae, Klots (1931/32) restricted the number of taxa and subdivided the genus into four subgenera. Of the European taxa, he arranged *brassicae* L. in the subgenus *Pieris* and the taxa *napi* L., *rapae* L., *manni* Mayer and *ergane* Geyer in the subgenus *Synchloe* Hbn. (together with e.g. *callidice* Esp.). Bernardi (1947) also used the system of subgenera, but, in part, arranged the species in different subgenera than previous authors. In his system, the subgenus *Pieris* was divided into three species groups: *Pieris* 1 s. str. with *brassicae* (of the European fauna), *Pieris* 2 with *napi, rapae, manni* and *ergane* and *Pieris* 3 which is not represented in Europe. Kudrna (1974), following Verity (1947), suggested that the species group which includes *napi, bryoniae* Ochsenheimer, *ergane, manni, rapae* and *krueperi* Staudinger should be placed in a separate genus, *Artogeia* Verity, and that *Pieris* only houses *brassicae, cheiranth* Huebner, *deota* Niceville and *brassicoides* Guerin-Meneville. However, Forster and Wohlfahrt (1976) claimed that the taxa included in *Artogeia* and *Pieris* are, in fact, congeneric.

3) Problems with European taxa at the species and subspecies level are well illustrated by the taxa of the *Pieris* (*Artogeia*) *napi*-group, the status of which have been widely discussed. In the past, it has not been possible to determine the phylogenetic distances between many taxa of this group and it has been necessary to use complicated taxonomic constructions such as the super- and semispecies concepts of Lorkovic (1962), Varga and Toth (1968) and Bowden (1972). Theories on the evolution of this group are confused (Petersen, 1949; Varga and Toth, 1978; Bowden, 1972) while relationships between North American, Asiatic and European taxa remain uncertain (Bowden, 1962). One reason for this confusion is the great variability of morphological characters within very similar taxa (for the variability of wing markings of *napi* and *bryoniae* see Mueller and Kautz, 1939). Investigations of closely related taxa indicate that the variability in coloration seems to be strongly influenced by environmental factors (e.g. Koyler, 1966; Shapiro, 1975, 1977).

Table 2

Enzyme	Abbreviation	Buffer System	Enzyme Stain
Adenylate kinase (2 Loci)	AK-1 AK-2	1	Brewer, 1970
Glyceraldehyde-phosphate dehydrogenase	GAPDH	1	Harris and Hopkinson, 1976
Arginine kinase	APK	1	Scholl (1)
Malate dehydrogenase (2 Loci)	MDH-1 MDH-2	1	Harris ans Hopkinson, 1976*
Aldolase	ALD	1	ibid
Indophenol oxydase	IPO	1	Brewer, 1970*
Malic enzyme	ME	2	Harris and Hopkinson, 1976
Hexokinase	HK	1	ibid
Pyruvate kinase	PK	1	ibid
Glutamate-oxaloacetate transaminase (2 Loci)	GOT-1 GOT-2	1	ibid*
6-Phosphogluconate dehydrogenase	6-PGD	1	Brewer, 1970*
Fumarase	FUM	1	ibid*
Isocitrate dehydrogenase (2 Loci)	IDH-1 IDH-2	1	Ayala et al., 1972
Glutamate pyruvate transaminase	GPT	2	Harris and Hopkinson, 1976
Phosphoglucomutase	PGM	1	Brewer, 1970
Phosphoglucose Isomerase	PGI	1	Scholl et al., 1978

Remarks:

Buffer Systems : System 1: Tris-citric acid buffer (Ayala et al., 1972)
System 2: Tris borate EDTA buffer (Ayala et al., 1972)

*With minor modifications, but the original staining medium would also be sufficient

(1) Scholl (personal communication): Agar overlay method. Staining mixture: Glycin buffer (1.52 M Glycin, 42 mM $MgCl_2 \cdot 6\ H_2O$, pH 9.0): 5ml, PEP : 10 mg, ATP : 40 mg, $NADH_2$: 10 mg, LDH : 90 iU, PK : 20 iU

Discussions are also in progress on the *Euchloe "ausonia*-complex", especially the status of the monovoltine populations flying in the Alps and Pyrenees and the bivoltine populations of the lower part of France and Italy (Back, 1979).

Recently biochemical methods have become important in the analysis of phylogenetic relationships. Enzyme electrophoresis in particular has proved very informative (Ayala *et al.*, 1974; Avise, 1974; Selander and Johnson, 1973). In this method, the extent of genetic divergence is estimated from variation in electrophoretic mobilities of a sample of

homologous enzymes of the various taxa. This approach is basically a genetic method but differs from other methods, for example the determination of interspecific fertility-bridges or sterility-barriers by hybridization experiments, in that the degree of genetic divergence is estimated from a defined sample of the genotype. Again this is not the case when evaluation of phylogenetic relationships is based on morphological data.

It has been demonstrated that phylogenetic independence of a taxon is correlated with gradual biochemical-genetic divergence (Ayala *et al.*, 1974; Avise, 1974; Selander and Johnsen, 1973) manifested in an increasing number of electrophoretically distinguishable enzyme variants. Enzyme electrophoresis may provide a basis independent of environmental variation for assessing the evolution of the Pieridae. This paper reports

Table 1

Taxon	Number of animals	Countries of origin	Number of population samples (1)	Number of sampling sites (2)
Aporia crataegi L.	19	CH,F	1	2
Pieris brassicae L.	141	CH,F,D,IRL	9	17
Pieris cheiranthi Hbn.	16	E	1	1
Artogeia rapae L.	154	CH,F,I,D	9	17
Artogeia mannii Mayer	25	F	1	1
Artogeia napi napi L.	276	CH,F,I,D,GB	16	24
Artogeia napi bryoniae Hbn.	63	CH	4	4
Pontia daplidice L.	5	CH,F,I	0	4
Pontia callidice Hbn.	5	CH	0	1
Euchloe simplonia Freyer	11	CH	0	5
Euchloe crameri Btlr.	11	F	1	1
Euchloe tagis bellezina Bois.	13	F	1	1
Anthocharis cardamines L.	45	CH,F	3	10
Anthocharis euphenoides Staud.	11	F	1	1
Colias phicomone Esp.	78	CH	5	5
Colias palaeno europome Esp.	60	CH	4	4
Colias myrmidone Esp.	6	D	0	1(3)
Colias crocea Geoff.	9	I	0	1
Colias hyale L.	5	D	0	1(3)
Colias australis Vrty	7	CH,D,F	0	4
Catopsilia florella F.	4	E	0	1
Gonepteryx rhamni L.	6	CH	0	1
Gonepteryx cleopatra L.	7	F	0	1
Leptidea sinapis L.	13	CH,F	1	1

Remarks :

Code for countries: CH = Switzerland, D = Federal Republic of Germany, E = Spain, F = France, GB = Great Britain, I = Italy IRL = Republic of Ireland

(1) a population sample consists of a minimum of 10 individuals from each sampling site

(2) this includes samples with less than 10 individuals

(3) laboratory reared

electrophoretic investigations of European taxa. A more comprehensive study, incorporating non-European material, is in preparation.

Materials and Methods

The material used in this study is listed in Table 1. Samples (only adults were studied) were usually stored at -30°C until electrophoresis. Control experiments showed that the electrophoretic mobility of the enzymes is identical in extracts from deep frozen and fresh specimens. There was however a considerable loss of GAPDH and ALD activity (for abbreviations used see Table 2) in frozen butterflies.

Supernatant fractions were prepared for electrophoresis from homogenates of an individual's thorax and head or its abdomen. The enzymes ME and GPT were assayed in supernatants from abdominal homogenates and other enzymes in supernatants from homogenates of the thorax and head. All enzymes were electrophoresed on vertical starch gels using standard procedures of our laboratory which have been described in detail elsewhere (Scholl *et al.*, 1978).

The enzymes assayed are listed in Table 2. A total of 20 enzyme loci has been scored in all taxa, with the exception of *Euchloe tagis bellezina* and *Leptidea sinapis*, for which only 18 enzyme loci were studied.

All taxa have been compared directly with each other. A precise characterization of all electrophoretic variants (electromorphs) was obtained by repeated cross-comparisons, with the exception of some rare variants. For each enzyme the designation of electromorphs (Tables 3.1 and 3.2) was made by comparison to the most frequent variant of *Pieris brassicae*, which defined the index 100. Other electromorphs were designated by measuring the difference (in millimeters) in their mobility compared to that of the reference *P. brassicae* variant (under our conditions of electrophoresis) and then adding or subtracting this value from 100. Thus IDH 103 refers to an IDH-electromorph that migrates 3 mm faster than the most frequent IDH-variant of *P. brassicae*.

The correct genetic interpretation of electrophoretically detectable enzyme phenotypes in polymorphic enzyme systems was proved by mating experiments (for comments see also Scholl *et al.*, 1978).

To estimate the genetic similarities between the taxa, the statistic $\bar{I}$ as defined by Nei (1972) was used. The similarity values $\bar{I}$ are presented in comparisons between pairs of taxa (Table 4) and are a measure of the proportion of electrophoretically identical proteins.

The similarity values $\bar{I}$ (Tables 4.1, 4.2, 4.3 and 5) were used to generate a dendrogram (Fig. 2) according to the unweighted pair) group average clustering method (Ferguson, 1980).

Results

The different electromorphs found at the various enzyme loci are presented in Tables 3.1 and 3.2. We have observed considerable

Figure 1

GOT-1 Zymogram

Intra- and interspecific variability of the enzyme pattern

Species	Genotype
Euchloe crameri	112/118
"	103/112
Euchloe simplonia	103/103
"	"
Pontia daplidice	85/85
Anthocharis cardamines	93/93
"	"
Aporia crataegi	98/98
Pieris brassicae	100/100
Artogeia napi napi	80/90
"	90/90
"	"
"	"
"	"
"	"

GOT-1 heterozygote individuals show a 3-banded phenotype

polymorphism at some loci and this polymorphism was found in most taxa where a large number of individuals have been investigated. At each locus only the electromorphs found at the higest frequency are given in Tables 3.1 and 3.2. The rare variants which are not given do not affect the conclusions of the present work. Enzymes which exhibit a low degree of interspecific variation, i.e. which migrate the same distance in the majority of species, are presented in Table 3.1. Enzymes which have greater interspecific variability are shown in Table 3.2. Since the designation of the electromorphs is based on the electrophoretic mobility of the enzymes, it can be seen from Table 3.1 and 3.2 that the relationships obtained from the enzyme pattern agree well with the taxonomic classification obtained with classical systematic methods. Electrophoresis patterns are more similar between congeneric species than between species of different genera. Electrophoretic identity can only be found in some isolated cases between species of different subfamilies. It is of interest that three enzymes (AK-1, AK-2 and GAPDH) are invariant in all taxa investigated.

This correlation between genetic similarity, assessed from enzyme variants and taxonomic classification becomes clearer if the comparison is based on coefficients of genetic similarity rather than the enzyme pattern. These coefficients are listed in Tables 4.1, 4.2 and 4.3 as comparisons between pairs of the taxa of the subfamilies Pierinae, Anthocharinae and Coliadinae, respectively. The subfamily Dismorphiinae is not listed, since only one species (*Leptidea sinapis*) was studied.

Table 3.1 : Electromorphs found at highest frequency in each species. Enzymes with low interspecific variation

Taxon	AK-1	AK-2	GAPDH	APK	MDH-1	ALD	IPO	ME	HK	GOT-2
A. crataegi	100	100	100	94	100	100	102	100	106	93
P. brassicae	100	100	100	100	100	100	100	100	100	100
P. cheiranthi	100	100	100	100	100	100	110	100	100	100
A. rapae	100	100	100	100	100	100	102	98	100	100
A. manni	100	100	100	100	100	100	102	98	100	100
A. n. napi	100	100	100	100	100	100	102	103	110	100
A. n. bryoniae	100	100	100	100	100	100	102	103	110	100
P. daplidice	100	100	100	94	100	100	96	104	100	100
P. callidice	100	100	100	94	89	100	96	100	98	100
E. simplonia	100	100	100	100	100	100	112	100	98	100
E. crameri	100	100	100	100	100	100	112	100	98	100
E. tagis bel.	100	100	-	94	100	-	112	99	103	100
A. cardamines	100	100	100	94	89	100	112	100	104	98
A. euphenoides	100	100	100	100	89	100	112	103	104	98
C. phicomone	100	100	100	94	100	100	103	103	123	95
C. palaeno	100	100	100	94	100	100	103	103	116	95
C. myrmidone	100	100	100	94	100	100	103	103	116	93
C. crocea	100	100	100	94	100	100	103	99	116	97
C. hyale	100	100	100	94	100/110	100	103	99	123	95
C. australis	100	100	100	94	100	100	103	99	116	95
Cat. florella	100	100	100	94	100	100	108	95	112	98
G. rhamni	100	100	100	94	89	85	103	99	106	97
G. cleopatra	100	100	100	94	89	85	103	99	106	92
L. sinapis	100	100	-	100	100	92	-	97	106	85

The averages of similarity coefficients and their standard deviations found between the taxa of the different subfamilies are presented in Table 5 (as indicated in the method section, the calculation of these coefficients is based on the allele frequencies observed, including the minor alleles at polymorphic loci, which are not shown in Tables 3.1 and 3.2).

High similarity coefficients were obtained between taxa which are regarded as closely related from morphological and other data (e.g. between the members of the genus *Colias*). These taxa clearly show less similarity to more distantly related taxa (e.g. comparison of the *Colias* and *Gonepteryx* species). The lowest similarity coefficients are found between members of the different subfamilies.

The similarity coefficients between the Pierinae and Anthocharinae are clearly higher than between these two subfamilies and the members of the Coliadinae and *Leptidea sinapis*, the only species of the Dismorphiinae

Table 3.2 : Electromorphs found at highest frequency in each species. Enzymes with high interspecific variation

Taxon	MDH-2	6-PGD	FUM	IDH-1	IDH-2	PK	GOT-1	GPT	PGI	PGM
A. crataegi	98	93 / 100	105	93	90 / 100	112	98	102	90 / 96	111 / 115
P. brassicae	100	100	100	100	100	100	100	100	100	100
P. cheiranthi	100	93	100	100	100	100	100	100	100	95
A. rapae	90	107	98	95	110	106	88	100	107	100
A. manni	90	100	98	95	100	106	88	100	100	100
A. n. napi	90	100	90	98	100	100	90	115	100	102
A. n. bryoniae	90	100	90	98	100	100	90	115	100	102
P. daplidice	90	100	95	86	97	103	93	130	97	110
P. callidice	90	89	95	91	97	106	130	81	109	108
E. simplonia	102	87	104	78	90	103	103	126	108	90
E. crameri	102	93	104	78	90	103	112	121	108	100
E. tagis bel.	102	87	105	76	90	103	101	94	97	92
A. cardamines	91	92	105	84	97	103	93	118	91	85
A. euphenoides	91	103	105	84	97	103	93	104	82	92 / 100
C. phicomone	100	79	106	88	102	102	105	81	90	109
C. palaeno	100	79	106	88	102	102	105	88	80	102
C. myrmidone	100	79	106	88	102	100	105	98	98	109
C. crocea	100	79	106	88	102	100	105	88	107	109
C. hyale	95	79	93	88	102	95	105	81	98	109
C. australis	100	79	106	88	102	95	105	81	98	102 / 109
Cat. florella	92	79	106	88	108	92	105	81	96	109
G. rhamni	92	79	110	97	142	95	115	89	80	115
G. cleopatra	92	79	110	92 / 97	140	95	122	91	80	120
L. sinapis	97	96	97	90	120	115	113	113	114	129

investigated. *Leptidea sinapis* has the lowest similarity coefficients with all other taxa.

A dendrogram (Fig. 2) has been constructed from the similarity coefficients (Tables 4.1, 4.2, 4.3 and 5), computed from the primary data (Tables 3.1 and 3.2). It showss, more clearly, the grouping of the taxa based on the biochemical-genetic data. The four subfamilies can be recognized as four branchings, however it is remarkable that the Anthocharinae branch from the Pierinae. Branch points of the genera are usually above the level of subfamilies (between 0.36 and 0.59). *Colias* and *Gonepteryx* species branch at a higher level of genetic similarity than nongeneric species of the subfamilies Pierinae and Anthocharinae.

Discussion

It has become evident from numerous investigations in recent years that electrophoretic techniques provide a very valuable tool for systematisists.

Table 4.1 : Coefficients of genetic similarity ($\bar{I}$-values) between Pierinae-species.

	P. brassicae	P. cheiranthi	A. rapae	A. manni	A. n. napi	A. n. bryoniae	P. daplidice	P. callidice
A. crataegi	.35	.32	.33	.35	.34	.34	.37	.32
P. brassicae		.88	.53	.64	.56	.57	.42	.32
P. cheiranthi			.52	.58	.51	.51	.37	.32
A. rapae				.90	.51	.56	.43	.38
A. manni					.60	.60	.47	.38
A. n. napi						1.0	.42	.32
A. n. bryoniae							.42	.32
P. daplidice								.55

Table 4.2 : $\bar{I}$ - values between Anthocharinae-species

	E. crameri	E. tagis bell.	A. cardamines	A. euphenoides
E. simplonia	.83	.52	.36	.37
E. crameri		.48	.37	.39
E. tagis bell.			.40	.39
A. cardamines				.74

The application of these techniques in establishing genetic relatedness, however, appears to be restricted mainly to congeneric species (Ayala *et al.*, 1974; Avise, 1974; Selander and Johnson, 1973), since the range of similarity detectable by enzyme-electrophoresis rapidly decreases beyond the generic level and reaches the critical point where two species have no electrophoretically identical enzymes in common. It is of interest therefore that in the Pieridae it has been possible to clearly establish genetic affinities even in interspecific comparisons of members of different subfamilies. It should be emphasized, however, that the sample of enzymes investigated has some influence on the similarity coefficients,

since rates of divergence are quite different for individual enzymes. It is possible that in this study a rather conservative sample of enzymes has been investigated.

Three enzymes, AK-1, AK-2 and GAPDH, were almost invariant in all Pierid species investigated and were the main, though not exclusive contributors, to the level of genetic identity found in inter-subfamilial comparisons. These three enzymes may prove very valuable for enzymatic characterization of butterfly taxa beyond the generic level, though further work will be necessary to establish this point.

Table 4.3 : $\bar{I}$ - values between Coliadinae-species

	C. palaeno europ	C. myrmidone	C. crocea	C. hyale	C. australis	Cat. florella	G. rhamni	G. cleopatra
C. phicomone	.85	.79	.71	.76	.83	.62	.35	.34
C. palaeno europ.		.79	.77	.63	.79	.53	.35	.35
C. myrmidone			.82	.63	.79	.59	.34	.34
C. crocea				.64	.80	.54	.40	.39
C. hyale					.82	.56	.45	.44
C. australis						.64	.44	.64
Cat. florella							.33	.33
G. rhamni								.78

Table 5 : Mean $\bar{I}$-values and standard deviations between the taxa of the different subfamilies

	Anthocharinae	Coliadinae	Dismorphiinae (L. sinapis)
Pierinae	0.33 ±0.07	0.28 ±0.06	0.22 ±0.03
Anthocharinae		0.25 ±0.04	0.22 ±0.07
Coliadinae			0.17 ±0.03

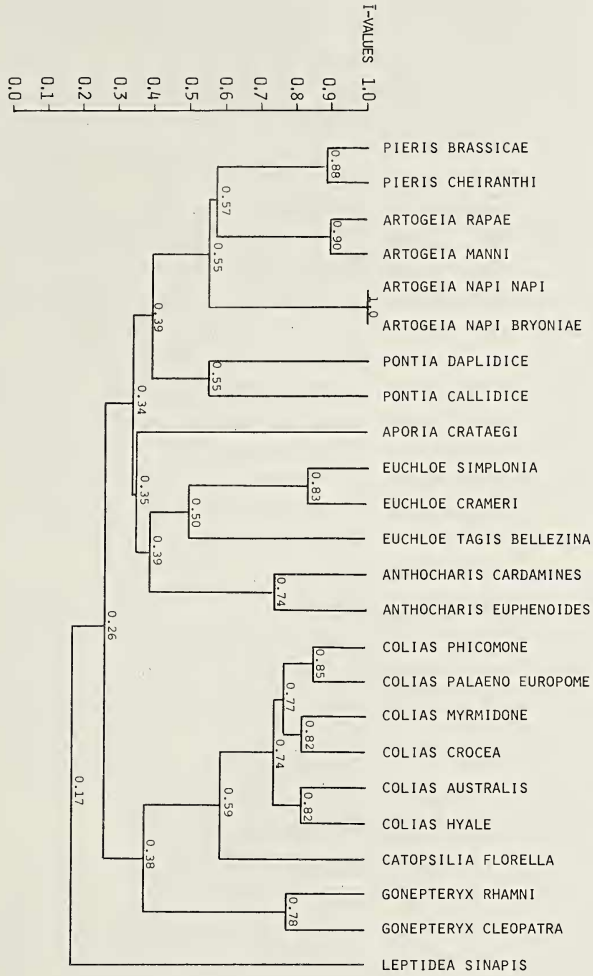

Figure 2.: Dendrogram of 24 European Taxa

1. The genetic divergence of the subfamilies

The subfamilies investigated show different degrees of genetic divergence (Table 5). *Leptidea sinapis*, the only Dismorphiinae in this study, is clearly isolated by the biochemical-genetic criteria as it has the lowest similarity coefficients. The genetic similarity between the Pierinae and Anthocharinae is greater than between these two subfamilies and the Coliadinae. The Anthocharinae may be characterized biochemically as a unit, but their branching point from the Pierini lies at the same level as those between different genera of the Pierini or that between the genera

Gonepteryx and *Colias* (Fig. 2). Therefore this biochemical data support the decision of Ehrlich (1958) and others to group the Anthocharini and Pierini as tribes of the Pierinae.

The somewhat closer electrophoretic similarity of *Aporia crataegi* to the Anthocharinae than to the Pierinae is probably not significant, but supports the relatively close biochemical-genetic relationship between the two subfamilies.

The isolated position of *Leptidea sinapis* results from the observation that this species has common electromorphs with other Pieridae only at the AK-1, AK-2, APK and MDH-1 loci which are all highly conservative, i.e. have a low number of electromorphs in the material investigated (see Table 3.1). Lorkovic recently expressed some doubts, based on morphological observations, as to whether *Leptidea* is really a Pierid (personal communication). It would be of interest to investigate whether the low degree of genetic similarity of *Leptidea sinapis* is typical for other taxa of the subfamily Dismorphiinae or the genus *Leptidea*. At the same time, however, the genetic divergence of species of other butterfly families from the Pieridae should be established and compared with the value found for *Leptidea sinapis*.

2. The position of *Catopsilia florella* F. 1775

There is a remarkable high genetic similarity between *Catopsilia florella* and species of the genus *Colias*. This similarity is clearly higher than that between species of *Colias* and *Gonepteryx*. Higgins (1975) classified *Catopsilia florella* as a Pierinae species. The biochemical genetic data support the opinion of most other authors (e.g. Klots, 1931/32), that *Catopsilia florella* should be included together with the species of the genera *Colias* and *Gonepteryx* into the subfamily Coliadinae.

3. The division of the genus *Pieris* Schrank 1801

Verity (1947) and Kudrna (1974) maintain that the genus *Pieris* Schrank 1801 should be subdivided into two genera, *Pieris* and *Artogeia* Verity 1947 respectively. The taxa *brassicae* and *cheiranthi*, which were thus isolated in the "new genus *Pieris*", show the same biochemical-genetic similarity with taxa of the "new genus *Artogeia*" as is found between *Artogeia* taxa themselves. Therefore, the electrophoretic data do not support the proposal that the old genus *Pieris* should be split in two.

Current investigations of additional taxa of this group may clarify the phylogenetic relationships.

4. The relationships between the taxa *brassicae* L. and *cheiranthi* Hbn. and between the taxa *simplonia* F. and *crameri* Btlr.

Unlike sympatric species where reproductive isolation is directly demonstrable, the relationships of allopatric populations is often difficult to determine. *Pieris brassicae* L. and its isolate *P. cheiranthi* Hbn. in the Canary Islands, and *Euchloe crameri* Butler of the Mediterranean region

and its subalpine relative *E. simplonia* Freyer are examples of such cases. *P. cheiranthi* was originally described as a new species, but has since been regarded by many authors as a subspecies of *P. brassicae* (e.g. Higgins, 1975). Others maintain that they are specifically distinct (Kudrna, 1973; Schurian, 1975). Similarly, *E. simplonia* is often considered as a "glacial" subspecies conspecific with *E. crameri*, even though many others have assumed them to be good species on the basis of morphological differences. In particular, distinctness of their larvae and pupae is well known (e.g., Catherine, 1920; Verity, 1947; Back, 1979). Neither enzyme analysis nor morphological studies measure reproductive relationships. Nevertheless, the magnitude of genetic divergence between related allopatric forms may provide some insight into such systematic problems when it is compared with data for the sympatric species pairs. The pairs *A. rapae* and *A. mannii, A. cardamines* and *A. euphenoides, G. rhamni* and *G. cleopatra*, and those of the genus *Colias* are at least partially sympatric. Some are sibling species. Similarity indices for these pairs range from 0.74 to 0.90. Some of these species have wide distribution ranges, but the genetic divergences in geographically distant populations so far studied are significantly lower than the range given above; for instance, *A. rapae* from Europe and Japan, or *A. cardamines* from Europe and Japan have the $\bar{I}$ value of at least 0.95 (unpublished data). In contrast, the $\bar{I}$ value of 0.88 for *E. crameri* and *E. simplonia*, falls within the range of genetic disparity found for sympatric species pairs. The interspecific genetic similarities of some sibling species may overlap with intersubspecific similarities in *Drosophila* (Ayala *et al.*, 1974). We have not encountered similar cases in butterflies, yet future studies may reveal such situations. With this reservation, therefore, the data suggest specific distinctness of the above allopatric pairs. Rapid genetic drift and establishment of reproductive barriers in island populations or experimental stocks founded from small numbers of individuals have been demonstrated in *Drosophila* (e.g. Carson, 1968; Templeton, 1980). *P. cheiranthi* may represent such an example. It would have been extremely interesting if the genetic composition of the newly founded colony of *P. brassicae* in Chile (Gardiner, 1974) were followed from the beginning and compared with *P. cheiranthi* and European *P. brassicae*.

5. The *napi - bryoniae* problem

The taxa *napi* L. and *bryoniae* Hbn. have been regarded as: different species (Mueller and Kautz, 1938; Forster and Wohlfahrt, 1976), as semispecies (Lorkovic, 1962) or as subspecies (Higgins, 1975). Bowden (1972) describes the holarctic *Pieris napi - bryoniae* complex as a "perfect example of a superspecies". The present results on 20 enzyme loci support previous observations (Geiger, 1978) that biochemical-genetic differences between these two taxa are extremely low ($\bar{I}$ values between population samples of at least 0.992). The same electromorphs occur in

both taxa, when very infrequent electromorphs are disregarded. The previous observations (Geiger, 1978) that differences between *napi* population samples are sometimes greater than differences between *napi* and (Swiss) *bryoniae*, has been substantiated here with more animals. A specific report on *napi* and *bryoniae* and on some closely related taxa is in preparation.

Acknowledgments. I am greatly indepted to PD Dr. A. Scholl, Zoological Institute of the University of Bern, for his generous support of the present work and for critically reading this manuscript. Comments and suggestions of Dr. I. Nakamura, New York, considerably helped to improve this paper. I am also grateful to Dr. Ch. Webb (University of Bristol, England) and Drs. F. and B. Westley for reading the English versions of the manuscript and to Mrs. L. Frauchiger and Mrs. V. Siegried (all of the Zoological Institute of the University of Bern) for assistance in the electrophoretic investigations. This work would not have been possible without the help of the following persons that sent me living material: Mr. R. Adams, F.R.E.S., Canary Islands (*P. Cheiranthi, C. florella*); Mr. S. R. Bowden, St. Albans, England (*A. napi*); Mr. G. Flauger, Teublitz, Germany (*C. australis, C. hyale, C. myrmidone*) and Dr. L. Rezbanyai, Museum of Nature Lucerne, Switzerland (*A. n. bryoniae*).

Literature Cited

AVISE, J. C., 1974. Systematic value of electrophoretic data. Syst. Zool. 23: 465-481.

AYALA, F. J., J. R. POWELL, M. L. TRACEY, C. A. MOURAO & S. PEREZ-SALAS, 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics 70: 113-139.

AYALA, F. J., M. L. TRACEY, D. HEDGECOOCK & R. C. RICHMOND, 1974. Genetic differentiation during the speciation process in *Drosophila*. Evolution 28: 576-592.

BACK, W., 1979. Zur Biologie der europaischen und nordwest-afrikanischen Populationen von *Euchloe ausonia* Huebner 1804. Atalanta X: 225-243.

BERNARDI, G., 1947. Revision de la classification des especes holoarctiques des genres *Pieris* Schr. et *Pontia* Fabr. Miscnea ent. 44: 65-79.

BREWER, G. J., 1970. An introduction to isozyme techniques. Academic Press, New York and London.

BOWDEN, S. R., 1972. *Pieris napi* L. (Pieridae) and the superspecies concept. J. Lepid. Soc. 26: 170-173.

_____., 1979. Subspecific variation in butterflies: Adaptation and dissected polymorphism in *Pieris* (*Artogeia*) (Pieridae). J. Lepid. Soc. 33: 77-111.

CARSON, H. L., 1968. *In* "Population Biology and Evolution" (ed. R. C. Lewontin, 1968).

CATHERINE, G., 1920. Notes sur *Anthocharis belia* Cr. et *ausonia* Hb. du departement du l'Aube. In Oberthuer, C., Etud. Lepid. Comp. 17, pp. 48-53. Corrigenda by Oberthuer, id. 17 (Planches), p. 46.

EHRLICH, P. R., 1958. The comparative morphology, phylogeny and higher classification of the butterflies (Lepidoptera: Papilionoidea). Univ. Kansas Sci. Bull. 39: 305-370.

FORSTER, W. & T. WOHLFAHRT, 1976. Die Schmetterlinge Mitteleuropas, 2. Tagfalter. Franckh, Stuttgart.

FORD, E. B., 1945. Butterflies. London. Collins.

FERGUSON, A., 1980. Biochemical Systematics and Evolution. Blackie, Glasgow and London.

GARDINER, B. O. C., 1974. *Pieris brassicae* L. established in Chile; another palearctic pest crosses the atlantic (Pieridae). J. Lepid. Soc. 28(3): 269-277.

GEIGER, H. J., 1978. Die systematische Stellung von *Pieris napi bryoniae*: Biochemisch-genetische Untersuchungsbefunde (Lep.: Pieridae). Ent. Z. 88: 229-235.

HARRIS, H. & D. A. HOPKINSON, 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland, Amsterdam and Oxford.

HIGGINS, L. G., 1975. The classification of european butterflies. Collins, London and Glasgow.

KLOTS, A. B., 1931/32. A generic revision of the Pieridae (Lepidoptera). Ent. Am. 12 (n.s.) (3-4): 139-242.

KOYLER, J. M., 1966. The effect of certain environmental factors and chemicals on the markings of *Pieris rapae* (Pieridae). J. Lepid. Soc. 20: 13-27.

KUDRNA, O., 1973. On the status of *Pieris cheiranthi* Huebner (Lep., Pieridae). Ent. Gazette 24: 299-304.

_____, 1974. *Artogeia* Verity, 1947, Gen. rev. for *Papilio napi* Linnaeus (Lep. Pieridae). Ent. Gazette 25: 9-12.

LORKOVIC, Z., 1962. The genetics and reproductive isolating mechanism of the *Pieris napi bryoniae* group. J. Lepid. Soc. 16: 5-19, 105-127.

MUELLER, L. & H. KAUTZ, 1939. *Pieris bryoniae* O. und *Pieris napi* L. Osterr. ent. Ver. Wien.

NEI, M., 1972. Genetic distance between populations. Amer. Natur., 106: 283-292.

PETERSEN, B., 1949. On the evolution of *Pieris napi* L. Evolution III: 269-278.

ROEBER, J., 1908. Pieridae in Seitz, A.: Die Gross-Schmetterlinge der Erde. Vol. 5: Die Amerikanischen Tagfalter. Kernen, Stuttgart.

SCHOLL, A., B. CORZILLIUS & W. VILLWOCK, 1978. Beitrag zur Verwandtschaftsanalyse altweltlicher Zahnkarpfen der Tribus Aphaniini (Pices, Cyprinodontidae) mit Hilfe elektrophoretischer Untersuchungsmethoden. Z. zool. Syst. Evolut.-forsch. 16: 116-132.

SCHURIAN, K., 1975. Bemerkungen uber *Pieris cheiranthi* (Lep., Pieridae). Ent. Z. 85: 252-256.

SELANDER, R. K. & W. E. JOHNSON, 1973. Genetic variation among vertebrate species. Annual Review of Ecology and Scstematics 4: 75-91.

SHAPIRO, A. M., 1975. Photoperiodic control of development and phenotype in a subarctic population of *Pieris occidentalis* (Lepidoptera: Pieridae). Can. Ent. 107: 775-779.

_____, 1977. Phenotypic induction in *Pieris napi* L.: role of temperature and photoperiod in a coastal California population. Ecological Entomology 2: 217-224.

TEMPLETON, A. R., 1980. Modes of speciation and inferences based on genetic distances. Evolution 34(4): 719-729.

VARGA, Z. & I. TOTH, 1978. Obersicht der taxonomischen und chorologischen Verhaltnisse in der *Pieris (Artogeia) napi-bryoniae*-Gruppe (Lep.: Pieridae) mit Ruecksicht auf die Anwendung des Superspezies-Begriffes in der Lepidopteren-Taxonomie. Acta biol. Debrecina, 15: 297-322.

VERITY, R., 1947. Le farfalle diurne d'Italia. Vol. III Firenze.